

Comparative Amino Acid Sequence Analysis of VP4 for VP7 Serotype 6 Bovine Rotavirus Strains NCDV, B641, and UK

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In a previous study (S. Zheng, G. N. Woode, D. R. Melendy, and R. F. Ramig, *J. Clin. Microbiol.* 27:1939-1945, 1989), it was predicted that the VP7 serotype 6 bovine rotavirus strains NCDV and B641 do not share antigenically similar VP4s. In this study, gene 4 and the VP7 gene of B641 were sequenced, and the amino acid sequences were deduced and compared with those of NCDV and bovine rotavirus strain UK. Amino acid sequence homology in VP7 between the three strains was >94%, confirming their relationship as VP7 serotype 6 viruses. VP4 of B641 showed amino acid homology to UK of 94% but only 73% homology to NCDV. Sequence comparison of a variable region of VP8 demonstrated amino acid homology of 53% between B641 and NCDV, whereas B641 and UK were 89% homologous in this region. These results confirm the earlier prediction that although the same serotype by VP7 reactivity, B641 and NCDV represent different VP4 serotypes. This difference in VP4 may have contributed to the lack of homotypic protection observed in calves, implicating VP4 as an important antigen in the active immune response to rotavirus infection in bovines.

Classification of group A rotaviruses into 11 serotypes has been based on the antigenic properties of outer capsid glycoprotein VP7 (reviewed by Estes and Cohen [3]). The viruses can be serotyped also from the properties of the other outer capsid protein, VP4. Both VP7 and VP4 stimulate the production of neutralizing antibodies, and sequence analyses of gene 4 and neutralizing reactivities of VP4-specific monoclonal antibodies indicate that VP4 serotypes segregate independently of VP7 serotypes (1, 2, 9, 12, 20, 24). In a recent study of the prevalence of different VP4 serotypes of human strains of rotavirus (9), it was demonstrated that virus strains with amino acid homologies of >89% in VP4 showed similar reactions with polyclonal antisera prepared with baculovirus-expressed VP4 antigens. This degree of homology was used to define VP4 serotypes.

Most bovine group A rotaviruses have been assigned to VP7 serotype 6 or 10. Two studies have compared the frequencies of infection of calves with these serotypes. In the first study (28), 89% of the isolates were placed in serotype 6 and 7.4% were placed in serotype 10 (B223 virus); in the second study (25), the figures were 66 and 7.4%, respectively. There is only limited information as to the different VP4 serotypes of bovine rotaviruses. There are at present two confirmed VP4 serotypes in the bovine rotavirus population, represented by the VP7 serotype 6 strains UK and NCDV.

In a previous study (28), the VP7 serotype 6 bovine rotavirus strains NCDV and B641 failed to demonstrate homotypic protection in calves. In a subsequent study (30), these two strains, along with the VP7 serotype 10 strain B223, were further characterized antigenically by monoclonal antibody reactivities. VP7 and VP4 of B223 were shown to be antigenically distinct from B641 and NCDV. Although the VP7s of NCDV and B641 were shown to be similar on the basis of polyclonal and monoclonal antibody reactions, on the evidence of reactivity of a B641 VP4-specific mono-

clonal antibody, it was suggested that NCDV and B641 may not share the same gene 4. It was also suggested that different VP4s may have contributed to the lack of homotypic protection between the two strains that was observed in calves.

To further define the relationship between these bovine rotavirus strains, gene 4 and the VP7 gene of B641 were sequenced, and the deduced amino acid sequences were compared with published sequences of VP4 and VP7 of NCDV (5, 22) and UK (2, 14).

B641 rotavirus was grown in BSC-1 cells as described previously (29). Dideoxynucleotide sequencing was performed directly on mRNA produced from purified single-shelled particles in an *in vitro* transcription system by methods previously described (4, 6). Synthetic oligonucleotide primers complementary to the mRNA of gene 4 of bovine rotavirus UK (14) and the VP7 gene of NCDV (5) were used at intervals of 200 to 300 nucleotides to sequence these genes in their entirety. To obtain the sequence at the extreme 3' end of the mRNAs, positive-sense oligonucleotide primers corresponding to sequences approximately 50 nucleotides upstream from the 3' end of each gene were used to sequence the minus strand of double-stranded RNA.

The complete nucleotide sequences for the VP7 gene and the VP4 gene of B641 are given in Fig. 1 and 2, respectively. The comparative amino acid sequence analysis for VP7 is given in Fig. 3, and that for VP4 is shown in Fig. 4. The percent amino acid homologies are presented in Table 1. Comparison of the amino acid sequence of VP7 from rotavirus strains B641, NCDV, and UK revealed sequence homologies of >94%. This high degree of homology was to be expected since regions of VP7 that show divergence among strains appear to be conserved among viruses of the same serotype (5, 6, 11, 22). In regions of VP7 demonstrated to be associated with serotype specificity (3), A (amino acids [aa] 87 to 101), B (aa 143 to 152), and C (aa 208 to 221), homologies between the three strains ranged from 90 to 100% for each region, confirming their relationship as VP7 serotype 6 viruses. Amino acid sequence analysis of VP4

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1  GGCTTTAAAGCGAGAATTTCCGTTTGCTAGCGGTTAGCTCCTTTTAAATGATGGTATTGAATATACCACAATTCTAATCTTCTTGACATCGATTACAT
101 TGCTGAATTATACCTTAAATCAATAACGAGAATAATGGACTATATAATTTACAGATTTCTGCTTATAGTGGCAATCTTGGCCACCATAATGAATGCGCA
201 GAATTATGGAGTGAATTTGCCAATTACAGGTTCAATGGATACTGCGTATGCAAAATCTACGCAAAGTGAACCATTTTGGACATCAACTCTTTGTTGTAT
301 TATCCTGTTGAGGCATCAATGAAATGGCTGATACCGAATGGAAGATACCTTATCACAAATTATTCTTGACAAAAGGATGGCCAACAGGATCGGTGTACT
401 TTAAGAATATACTGATATAGCGGCTTTTTCAGTAGAACCACAGCTGTACTGTGATTATAATTTAGTTTTAATGAAATATGATTCTACACAGAAACTAGA
501 TATGCTGAATTAGCCGATCTCATACTGAACGAATGGCTGTGCAATCCAATGGACATAACACTGTATTATTATCAGCAGACTGATGAAGCAATAAATGG
601 ATATCGATGGGTTCTTCTTGACAGTCAAAGTGTGTCCGTTAAATACGCAGACACTTGGTATTGGATGTCTAATAACCAATCCAGACACGTTTGAAACAG
701 TTGCGACAACGGAGAAGTTGGTGATTACAGATGTTGTAGATGGTGTCAACCATAAGTTAAACGTCACAACAGCAACGTGCACCATACGCAACTGTAAAAA
801 GTTAGGACCAAGGGAGAACGTAGCAGTCATACAGGTAGGCGGCGCAAAATATTTAGACATCACAGCTGATCCAACAACACACCAGACAGAGAGGATG
901 ATGCGAATAAATTGGAATAAATGGTGGCAAGTGTCTACAGTGGTGGATTATGTCAATCAAATAATTCAAACGATGTCCAAAAGATCTAGATCGCTTA
1001 ATTCGTCAGCGTTCTATTACAGAGTATAGGTCATGTTAGATTAGAGTTGTATGATGTGACC

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FIG. 1. Complete nucleotide sequence of the VP7 gene of bovine rotavirus strain B641. Underlined bases indicate positions of initiation and termination codons.

showed low homology of 73% between B641 and NCDV, whereas B641 VP4 and UK VP4 were 94% homologous. These observations support the suggestion that B641 and NCDV, although the same serotype by VP7 reactivity, appear to represent different VP4 serotypes.

VP4 is cleaved by proteases to subunits VP8* and VP5*, enhancing rotavirus infectivity. Previous studies have demonstrated that cross-reactive neutralizing epitopes of VP4 are mostly present on the VP5* subunit, and those neutralizing epitopes that show more type specificity are located in the VP8* subunit (9, 18, 24, 27). Because of the degree of variability in VP8* among other rotavirus strains, a sequence comparison was made for aa 100 to 200, which represent part of a major variable domain and may include an immunodominant epitope in VP8* (8, 10). In this region, NCDV and B641 demonstrated only 53% homology. For B641 and UK, the homology for this region was 89%. Several sites on VP5*

involved in cross-reactive neutralization (aa 306, 388, 393, 434, and 440) have been located by sequencing escape mutants selected by cross-reactive monoclonal antibodies (18, 24, 26). VP5* of B641 and NCDV differ in three (aa 306, 393, and 440) of these sites.

The sequence data presented support the previous suggestion that B641 and NCDV are different VP4 serotypes. The differences observed, that is, low amino acid homology for all of VP4 and a major variable domain in VP8*, and amino acid differences at sites involved in cross-reactive neutralization on VP5*, may be contributing factors in the lack of homotypic protection between these two strains that was observed in calves, implicating VP4 as an important antigen in mediating the active immune response in bovines. These results and previous reports (14, 17) indicate that there are two confirmed VP4 serotypes present in the bovine population represented by UK, B641, B1, and B11 and by NCDV

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1  GGCTATAAAATGGCTTCGCTCATATACAGACAGCTGCTCGCTAATCTTACGAGTGGATTGTCTGATGAGATACAGTCAGTTGGGTACAGAGAAGAACC
101 AACCGGTTACAGTGGATCCAGGACCATTTGCGCAGACAGGATATGCACAGTGAATCGGGGCGCTGGTGAAGTGAATGATTGCTGTTGGTACAACCTGT
201 GTTGGATGGACCGTATCAACAGCGCCGTTGATCTACAGTAGGAAATCGGATGTTGTTAGCACCAACAGGACAGGTGTAGTAGTGGAAAGGACGGAC
301 AATTCTGGCAGATGGTTATCTGTAATTCTGATTGAACCGGTTGCATCATAGAGACTAGAACGTATACGATGTTTGGATCAAGTAAACAGGTGTAGTGT
401 CGAACGTGTCTGATACGAAATGGAATGTTTGAATGATGAAGACGGCGGTTGATGGTGACTATGCGGAATGGGGAACATTATTATCGGACATTAAT
501 CTACGGGATGATGAAATATGGAGAGAGATTATTCATATACGAAGGAGAAACCCCAATGCCAGAACCAAGGATACATTGTAAACGAATTATACATCAGTT
601 GAGGTAAGACCATATAGTGACTTCTATATAATTTCAAGATCGCAGGAATCAGCATGCACTGAGTACATAAACCAACGGACTGCCGCTATCCAAAATACCA
701 GGAATGTAAGTGCCTTTGGCGATATCATCCAGGTCAATTAACCAAGAAAAGTGCAGCCTAATGAAGATATTGTAGTTTCTAAGACTTCATTATGGAAGA
801 ATTGCAATACAATAGAGATATCATAATTAGATTTAGGTTTGATAACTCAATAATAAAGCTGGAGGTTTGGGCTATAAGTGGGCTGAAATCTCATTTAAA
901 GCTGCAAAATATCAATAAATACATAAGCGACGGAGAAGAGGTTACAGCGCATACGACGTGTTCAAGTTAATGGCGTTAATGATTTCAGCTTTAACGGAG
1001 GCTCGTTACCAACTGATTTCCGAATATCAGATATGAAGTAATTAAGAAAATTCGTATGTATACGTTTACTACTGGGATGATTACAGGCATTACAGAAA
1101 CATGGTCTACGTGCGATCATTGGCAGCGAATTTGAATGACGTAATGTGTTCTGGTGGAGATTATAGCTTCGCGCTAATTGTTGGACAGTGGCCGGTGATG
1201 AAAGGAGGGGCGGTAACGTTGCATACAGCAGGAGTAACATTATCTACACAATTCACCGACTTCGTATCGTTAACTCACTAAGGTTTAGGTTTAGACTGT
1301 CTGTAGAAGAACCGTCATTACAGATAACTAGAACCGGTATCAAACTGTATGGTTTACCAGCAGCGAACCAACGGCGGAAGGGAATATTATGAAGT
1401 GGCAGGAAGGTTTTCGCTCATATCATTGGTGCCATCAATGATGACTATCAACGCCAATATGAACCTCAGTAACAGTAAGGCAAGACTTGGAAGGCGT
1501 TTAATGAGTTGAGAGAAGAGTTTAATAACTTATCACAAGAAATAGCTGTGTCACAGTTAATTGACTTAGCTATCTGCCATTAGACATGTTTTCGATGT
1601 TCTCGGAATTGAGGGTACTGTGAACGCAGCAAAATCAATGGCTACCAACGTGATAAGGAAATTAAGAGCTCAAACTCGCATCATCAGTGTCAATGTT
1701 GACGGACTCTTTATCCGATCGGCGCTCATCTATTCAAGGAGTACATCAATACGATCAATAGGATCAACAGCATCAGCTTGGACTAATATTTCAAAACAG
1801 ACACAAGACGCTGTTAATGAAGTTGCGACGATATCATCAACTGTCAACAATAAGTGGGAAATTAAGATTGAAAGAAATTACGACTCAACAGAGAGGAA
1901 TGAGTTTCGATGACATATCAGCGCGGTGCTAAAAGCCAATAGACAGATCAATACAGGTGACAAAAATGCATTACCAGACGTCATCAGAGAGCGTC
2001 AGAGAAATTCATCCGTAATAGGGCGTATAGAGTGATAGACGGAGATGAAGCATTTGAAGCCAGCACTGATGGAAGATTTTCGCGTACAAGGTGGAACA
2101 CTTGAGGAAATGCCATTGATATAGAAAAATTTGAGATTTAGTTACTCGCTCACCAGTGATATCAGCAATAATAGACTTCAAGACGTTGAAAAACCTGA
2201 ATGACAATTATGGGATACTAGAGAGCAAGCATTTAATTTGTACGGTCAAACCCCAAGTTTTGCGTGGATTATGGACCAAAACAATCCAATTATAAA
2301 AAACAGGATAGAACAAATGATCATGCAATGTAGATTGTAGAGCTCTGGAGGATCTGACC

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FIG. 2. Complete nucleotide sequence of the VP4 gene of bovine rotavirus strain B641. Underlined bases indicate positions of initiation and termination codons.

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B641 1 MYGIEYTTILIFLTSITLLNYTLKISITRIMDYIIRFLLIVAILATIMNAQNYGVNLPITGSMDTAYANSTQSEPFLTSTLCLYYPVEASNEMADTEWKD
NCDV          I      M      V      I      D      I
UK          I      VV  MI      I

101  TLSQLFLTGWPTGSVYFKEYTDIAAFSVEPQLYCDYNLVLMYDSTQKLDMSELADLILNEWLCNPMDITLYYYQQTDEANKWISMGSSCTVKVCPLNT
          L  A      E      T
          E

201  QTLGIGCLITNPDTFETVATTEKLVITDVGDNHKLNVTTATCTIRNCKKLGPRENVAVIQVGGANILDITADPTTTPQTERMMRINWKKWQVFTTVV
          M      V      I      V      A

301  DYVNQIIQTMSKRSRLNSSAFYYRV

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FIG. 3. Amino acid sequence comparison of VP7 of serotype 6 bovine rotavirus strains B641, NCDV, and UK. Bold print represents regions associated with serotype specificity: A (aa 87 to 101), B (aa 143 to 152), and C (aa 208 to 221).

and C486. VP4s of the bovine rotavirus strains appear to be unique, as amino acid sequence analyses of bovine rotavirus strain VP4s with published sequences for VP4s of human and porcine rotavirus strains (7, 13–16, 18, 21–23) demonstrated homologies of <75%. On the basis of monoclonal antibody reactivity (30), it had been predicted that the VP7

serotype 10 strain B223 rotavirus may represent a third VP4 serotype, and a recent study confirms this prediction by plaque reduction neutralization of reassortants, showing VP4 of B223 to be distinct from NCDV and UK (19). Nucleotide sequence data for gene 4 of B223 have not yet been determined.

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B641 1 MASLIYRQLANSYAVDLSDEIQSVGSEKNQRTVDPGPFAQTGYAPVNRGPGEVNDSTVVQPVLDGPYQAPFDLPVGNRMLLAPTGGPVVVEGTDNSG
NCDV          T  T  E      EI  T  T  D  N      N      W  T  T  E      TT  NP  SYW      NA      N  TN
UK          N      G      N      W      W      W      R

101  RMLSVILIEPGVTSETRTYTMFGSSKQVLSVNSDTKMKFVEMTKTAVDGDYAEWGTLLSDIKIYGMKYERLFIYEGETPNATTGKYIVTNYTSVEVR
      AT      N  QQVE  L  QQV  T  D  Q      DLS  QTQ  N  SQH  S  TP  L  V  H  GKIYT  N      T  GY  ST  FDT  NMT
      A      M      V      V      T  L      R      FI  A  A

201  PYSDFYIISRSQESACTEYINNGLPPIQNTRNVVPLAISRRIKPRKVQANEDIVVSKTSLWKEMQYNRDIIRFRFNSIISKAGGLGYKWAEIFSKAAN
      A  C      PLA  AK      I  VS  V  N  VYTRA  P  Q      V  K  A      S      S  V  P
      V      E      K      S

301  YQYNYISDGEVTAHTTCSVNGVNDFSFNGGSLPTDFAISRVEIKENSYYVYVHYWDSQAFRNMVYVRSALANLNDVMCSGGDYSFALLVGQWPMKGG
      T  TR      I  NY      V  K      F  ID      N      D  S  T      P  NY  T
      MR      D      H      P

401  AVTLHTAGVTLSTQFTDFVSLNSLRFRRLAVEEPSFTITRTRVSKLYGLPAANPGGREYEVAGRFSLSLVPSNDDYQTPIMNSVTVRQDLERRLNE
      S  S      S  P  S  L  G      R  NSQ  I      I      Q  G
      K      F

501  LREEFNLSQEIAVSQLIDLAMLPLDMFSMFSGIEGTVNAAKSMATNVIRKFKSSKLASSVSMLTDSLSDAASSISRSTSIRSIGSTASAWTNISKQTQD
      D      Q  M      L      KS  ID      MKR  K  S  N  T  N      A  V  VS      EV  NITS
      S      M      A  V      A  E

601  AVNEVATISSQVSQISGKRLRLKEITTQTEGMSFDDISAAVLKAKIDRSIQVDQNALPDVITEASEKFIRNRAYRVIDGDFAFEASTDGRFFAYKVETLEE
      INVTSS  T  T  T  RR      MA  D  N      T  K  T  LNT  T  EIV      P      KD  VL  T  KY      F
      S      R      N      G

701  MPFDIEKFADLVTRSPVISAIIDFKTLKLNLDNYGITREQAFNLLRSNPKVLRGFDQNNPIIKNRIEQLIMQCRL
      I  VQ      D      S  Q  L      DR  E  ND  R  S
      NM      N      D

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FIG. 4. Amino acid sequence comparison of VP4 of bovine rotavirus strains B641, NCDV, and UK. Bold print represents a major variable region in VP8 (aa 100 to 200).

TABLE 1. Percent amino acid homologies in VP4, VP7, and VP8^a between B641, NCDV, and UK bovine rotaviruses

Virus	% Homology					
	UK			B641		
	VP4	VP7	VP8	VP4	VP7	VP8
NCDV	74	97	52	73	96	53
UK				94	97	89

^a Amino acids 100 to 200 of VP8.

Nucleotide sequence accession number. Nucleotide sequence accession numbers for B641 VP7 and VP4 genes are M63266 and M63267, respectively.

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